46. (New) The vaccine of claim 45 wherein said cytotoxin is formulated in admixture with an adjuvant.

47. (New) The vaccine of claims 46 wherein said adjuvant is an immunostimulating (ISCOM) matrix.

48. (New) The vaccine of claim 47 wherein said ISCOM matrix comprises *Quillaja* saponins, cholesterol and phospholipids.

49. (New) A recombinant protein depicted by the amino acid sequence SEQ ID NO: 6.

50. (New) A recombinant protein depicted by the amino acid sequence SEQ ID NO: 13.

REMARKS

This Amendment and Remarks are filed in response to the Office Action dated November 15, 2002 wherein all elected claims stand rejected on various grounds.

Election Restriction

Examiner withdrew claims 8-11, 16-22 and 26-33 from further consideration pursuant to 37 CFR 1.142(b) as being drawn to nonelected Groups II-XI, there being not allowable generic or linking claim.

Applicant's election with traverse of Group I, claims 1-7, 12-15 and 23-25, drawing to peptides, polypeptides, or a cytotoxin protein defined by SEQ ID NOs: 1 or 2, and specific peptides

encoded by SEQ ID NOs: 6 or 13, classified in class 530, subclass 350, in Paper No. 9, is acknowledged.

The traversal is on the ground(s) that "on the basis that at least with regard to Group I and III, the restriction is not proper", and states "Once Examiner searches the Group I for references to cytotoxin of *M. bovis*, any method for diagnosis or prophylaxis will necessarily be found and by the same correlation, when Group III or IV would be searched, *M. bovis* cytotoxin reference would be found. The claims of Groups I, III and IV are related as a composition and the use for such composition." The claims in Groups I, III and IV are asserted to not constitute a serious burden and are respectively requested to be examined at the same time. These arguments have been fully considered but are not found to be persuasive for the reasons below.

First, the classification system has no statutory recognition whether inventions are independent and distinct. For example, each class and subclass is comprised of numerous completely independent and distinct inventions.

Second, MPEP 803 states that restriction is proper between patentably distinct inventions where the inventions are (1) independent or distinct as claimed and (2) a serious search and examination burden is placed on the examiner if restriction is not required.

The term "distinct" is defined to mean that two or more subjects as disclosed are related, for example, as product and method of use, but are capable of separate manufacture, use or sale

as claimed, and are patentable over each other. In the instant situation, the inventions of Groups I, III and IV as well as Groups II, V-XI are drawn to distinct inventions which are related as separate products capable of separate functions. Restrictions between the inventions is deemed to be proper for the reason previously set forth.

In regard to burden of search and examination, MPEP 803 states that a burden can be shown if the examiner shows either separate classification, different field of search or separate status in the art. In the instant case a burden has been established in showing that the inventions of Groups I-XI are classified separately necessitating different searches of issued US Patents. However, classification of subject matter is merely one indication of the burdensome nature of search. The literature search, particularly relevant in this art, is not co-extensive, because for example genes, proteins, diagnostic fragments, and antigens would differ from method of using these products as the combination of reagents would differ. Additionally, it is submitted that the inventions of Groups I-XI have acquired a separate status in the art. Clearly different searches and issues are involved in the examination of each Group.

For these reasons the restriction requirement is deemed to be proper and is therefore made final.

The examiner is reading all of the claims as product by process claims, and the *Moraxella bovis* cytotoxin of claim 1 is not limited to a cytotoxin encoded by SEQ ID NO: 1. Claim 1 claims a

product, which can be produced by the recited process step of "produced" and the claimed product produced by a materially different process would anticipate the claimed invention.

Applicants disagree with Examiner's reasoning regarding the Restriction Requirement and reading all claims as product by process claims. At most, only claims 1-7 could be so read as they comprise some process steps. The other claims are clearly claims directed to the compositions. Examiner is requested to examine claims 12-15 and claims 34-50 as composition claims and not as product by process claims.

To expedite the examination, Applicants canceled the non-elected claims.

Rejections Under 35 USC §101

Claims 12-14 are directed to an amino acid sequence that is not isolated and purified; the claims do not show the "hand of man." The claimed invention is directed to non-statutory subject matter.

Applicants disagree, however, to meet Examiner's rejections, Applicants amended claims 12-14 to be directed to isolated and purified amino acid sequences, each sequence being claimed independently.

Rejections under 35 USC 112, First Paragraph

Claims 23-25 are rejected under 35 USC 112, first paragraph, because the specification while being enabling for the production of recombinant *Moraxella bovis* cytotoxin comprising SEQ ID NO: 2, and fragments of SEQ ID NO: 2, does not reasonably provide

enablement for the utilization of any fragments of SEQ ID NO: 2, to include SEQ ID NO: 6 or 13 as vaccines. The specification does not enable any person skilled in the art to which it pertains or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

The specification fails to teach how to formulate and use the claimed vaccines that comprises any fragment of SEQ ID NO:2. The term "vaccine" encompasses the ability of the specific antigen to induce protective immunity to treat or prevent infection or disease induction. The specification teaches a *Moraxella bovis* cytotoxin of SEQ ID NO: 2 that is able to induce a protective immune response when combined with an adjuvant.

The specification does not provide substantive evidence that the claimed vaccine that comprises any cytotoxin fragment or a composition without an adjuvant are capable of inducing protective immunity. This demonstration is required for the skilled artisan to be able to use the claimed vaccines for their intended purpose of preventing *Moraxella bovis* infections. Without this demonstration, the skilled artisan would not be able to reasonably predict the outcome of the administration of the claimed vaccines, i.e., would not be able to accurately predict if protective immunity has been induced.

The ability to reasonably predict the capacity of a single bacterial immunogen to induce protective immunity from *in vitro* antibody reactivity studies is problematic. Ellis exemplifies this

problem in the recitation that "the key to the problem (of vaccine development) is the identification of the protein component of a virus or microbial pathogen that itself can elicit the production of protective antibodies" (page 572, second full paragraph). Unfortunately, the art is replete with instances where even well characterized antigens that induce an *in vitro* neutralizing antibody response fail to elicit *in vivo* protective immunity. See Boslego et al., wherein a single gonococcal pillin protein fails to elicit protective immunity even though a high level of serum antibody response is induced. Accordingly, the art indicates that it would require undue experimentation to formulate and use a successful vaccine without the prior demonstration of vaccine efficacy.

Applicants disagree. However, in the interest of expediting examination of this application, Applicants canceled claims 23-25 and added new claims 34-40 directed to the vaccine comprising the purified recombinant cytotoxin of the amino acid sequence SEQ ID NO: 2.

Applicants new claims are supported in their entirety in Example 24.

Concerning the vaccines based on fragments of sequence SEQ ID NO: 2, identified as SEQ ID NOs: 6 and 13, Applicants canceled claims 24 and 25 and added new claims 41-48. Applicants wish to point out to the Examiner that the specification provides sufficient description and disclosure of the recombinant cytotoxin sequences SEQ ID NOs: 6 and 13 that by using the same procedure as

described in the Example 24, it would be easy and without undue experimentation to prepare vaccines comprising these cytotoxins. Contrary to the Examiner's argument, the specification teaches how to make the recombinant vaccine in Example 24 and on page 25, lines 16-21 of the specification, Applicants state "The recombinant vaccine is produced by identifying the relevant cytotoxin or a fragment thereof, cloning them and expressing them using suitable vectors. This approach yields immunogens which are pure and reproducible in sufficiently large quantities to allow preparation of a vaccine for active immunization."

The fragments are homologous to but have different amino acid sequence from other RTX toxin amino acid sequences which are known to be highly immunogenic. See Tables 4 and 5, page 15 of the specification, for amino acid sequences of the cytotoxins SEQ ID NOs: 6 and 13. Moreover, as Examiner admits, *in vitro* studies show production of high titre of antibodies. Since both fragments are isolated of the highly immunogenic protein of SEQ ID NO: 2, Examiner's scepticism regarding immunogenicity of these two protein is unwarranted.

Applicants respectfully request that Examiner reconsiders her rejection and allows the new claims 34-48.

Claims 12-15 and 23-25 are rejected under 35 USC 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The instant specification does not contain a written description of the invention in such full, clear, concise, and exact terms or in sufficient detail that one skilled in the art can reasonably conclude that applicant had possession of the claimed invention at the time of filing.

The claimed invention is directed to recombinant proteins (claim 15, and 23-25) that comprise an amino acid sequence fragment of SEQ ID NO: 2, or is encoded by one that comprises a fragment of SEQ ID NO: 1 (nucleic acid), an amino acid sequence that comprises fragment of SEQ ID NO: 2 (claims 12-14), or a recombinant protein that comprises an amino acid sequence of SEQ ID NO: 2, 6 or 13. What all of the DNA sequences that comprise a fragment of SEQ ID NO: 1, and comprise an amino acid fragment of SEQ ID NO: 2, as well as what all the proteins are that comprise any fragment of SEQ ID NO: 2, 6 or 13 from any source, and would serve as a recombinant vaccine have not been described.

The specification discloses recombinant proteins which may evidence alterations in the amino acid sequence, and which would evidence changes in the nucleic acid molecule but what alterations are in the claimed plurality of recombinant proteins, encoded by the recombinant genes that encode the plurality of proteins, that are not a *Moraxella bovis* cytotoxin, of SEQ ID NO: 2, encoded by SEQ ID NO: 1, or is a fragment of SEQ ID NO: 6 or 13, have not been described.

A description of a genus may be achieved by means of a recitation of a representative species, defined by nucleotide

sequence, falling within the scope of the genus or of a recitation of structural features common to members of the genus, which features constitute a substantial portion of the genus. Reagents of the University of California v. Eli Lilly & Co. 119 F3d 1559, 1569, 43 USPQ2d 1398-1412, 1406 (Fed. Cir. 1997).

The claimed amino acid sequences (claims 1-14), recombinant protein (claim 15), or recombinant cytotoxin that comprises an amino acid sequence fragment of SEQ ID NO: 2, or is encoded by a DNA that comprises a fragment of SEQ ID NO: 1, the products of which do not have any specific function but must only share a common amino acid sequence, have not been described.

The instant specification fails to provide sufficient descriptive information, such as definitive structural or functional features of the claimed genus recombinant proteins (claim 15) that have alterations in their gene loci (nucleic acid molecules that encode a plurality of proteins). Even if the claims were amended to require the encoded recombinant protein to evidence cytotoxic activity, only 4 species of the now claimed genus have been described and shown in figure 4-1.

There is no description of where or how the alterations must be made in the gene loci to achieve or maintain a hemolytic, leukotoxic or corneotoxic effect, or for the induction of a protective immune response upon administration of the recombinant protein to a host to protect against *Moraxella bovis* challenge, using any recombinant protein, that only shares a fragment of SEQ ID NO: 2, and is encoded by a gene that comprises only a fragment

of SEQ ID NO: 1.

The specification proposes to discover other members of the genus by using sequence homologies and introduction of alterations based upon what is already known. Altered genes that have structural features that could distinguish the claimed amino acid sequence and recombinant proteins from others excluded are missing from the disclosure.

Vas-Cath Inc. V. Mahurkar, 19 USPQ2d 1111, clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The inventions is, for purposes of the 'written description' inquiry, what is now claimed." (See page 1117). The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed." (See *Vas-Cath* at page 1116). Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 USC 112 is severable from its enablement provision. Reiger et al (glossary of Genetics and Cytogenetics, Classical and Molecular, 4th Ed., Springer-Verlay, Berlin, 1976) clearly define alleles as one of two or more alternative forms of a gene occupying the same locus on a particular chromosome....and differing from other alleles of that locus at one or more mutational sites (page 17). Thus, the structure of naturally occurring allelic sequences are not defined. Conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity

of the method of isolation. Adequate written description requires more than a mere statement that it is part of the invention and a reference to a potential method of isolating it. The nucleic acid itself is required.

Amino acid sequences that comprise a fragment of SEQ ID NO: 2 (claims 12-14), recombinant proteins that comprise a fragment of SEQ ID NO: 1 (claims 12-14), recombinant proteins that comprise a fragment of SEQ ID NO: 1 (claims 15 and 23-25), a recombinant protein homolog or allelic variant that shares a fragment sequence with SEQ ID NO: 2 and is able to induce a protective immune response against *Moraxella bovis* (claims 23-25) have not been described. Sufficient support for the generic claims has not been provided.

Applicants disagree, however taking in consideration Examiner's arguments, Applicants limited claims to solely those recombinant proteins which are specifically described, their sequences disclosed and their preparation and identification described in a great detail. Applicants also separated each entity into separate individual claims so as to avoid any possible inclusion of other non-claimed entities and in this regard, added two new claims 49 and 50 directed to recombinant proteins depicted by SEQ ID NOs: 6 and 13.

With this amendment, all variations alluded to by the Examiner are eliminated from the claims and rejections of claims 15 and 23-25 are overcome. Examiner is respectfully requested to reconsider the rejection and allow the newly amended claims.

Rejections Under 35 USC 112, Second Paragraph

Claims 5-7 are rejected under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 5 recites the phrase "a cytotoxin-enriched fraction". Claim 5 broadens the scope of claims 1-4 from which claim 5 depends, in light of the fact that claims 1-4 are directed to a purified or partially purified cytotoxin, and not an "enriched fraction" that comprises a plurality of cytotoxins. The word "fraction" lacks antecedent basis in claims 1-4. No column fractions were combined and diafiltrated. What is contained in the cytotoxin-enriched fraction? How does the enriched fraction of claim 5 further limit the purified cytotoxin of claims 1-4? An enriched fraction would be less pure than a purified composition; claim 5 does not further limit the purified compositions of claims 1-4.

Claim 6 is directed to a "hemolysin, leukotoxin or corneotoxin" and depends from claim 5. Claims 5 and 6 broaden the scope of claims 1-4 as three different types of cytotoxins are recited in claims 5-6. See Ruth Marrion, abstract page 4031-B that teaches Moraxella produces three different virulence factors, specifically a hemolysin, a cytotoxin and a leukotoxin.

Claim 7 defines the claimed cytotoxin to have a molecular weight of "about 95 and 98 kDa". How can the cytotoxin have two different molecular weights simultaneously? Are two different

molecules being claimed in claim 7, while claims 1-4 directed to a single cytotoxin? Claim 7 is broader in scope than claims 1-4 from which it indirectly depends.

Applicants disagree. However, to expedite the prosecution of this application, Applicants canceled claims 5 and 7 and amended claim 6. This amendment is believed to overcome rejections to the claim 6.

Rejections Under 35 USC §102

Claims 1-7, 12-15 and 23-25 are rejected under 35 USC 102(a) as being anticipated by WO 01/16172 A1, publication date 08 March 2001.

WO 01/16172 A1 discloses the claimed invention directed to a purified, partially purified or recombinantly produced *Moraxella bovis* cytotoxin, wherein the cytotoxin comprises the amino acid sequences of SEQ ID NO: 6, SEQ ID NO: 13, and is purified form culture supernatants, purified by centrifugation, and has biological activity of a hemolysin, leukotoxin or corneotoxin with a relative molecular weight of about 95 to 98 kDa (see Figure 6, page 5, lines 25-33; page 7, lines 29-36; pages 11-13, and 25-28) and sequence alignments attached hereto. WO 01/16172 A1 anticipates the instantly claimed invention.

Applicants disagree. Examiner argues that the current claims 1-7, 12-15 and 23-25 are anticipated by WO 01/16172, particularly with regard to sequences SEQ ID NO: 6 and SEQ ID NO: 13 and cites as evidence Figure 6 and page 5, lines 25-33, page 7, lines 29-36, pages 11-13 and pages 25-28.

Applicants respectfully point out that a) there is no Figure 6 in the cited reference; and b) page 5, lines 25-33, page 7, lines 29-36, pages 11-13 and pages 25-28 do not contain any sequence or refer to any specific sequence or a site within the sequence seen in Figure 5 which is being cited against the two current sequences SEQ ID NOs: 6 and 13. These sequences (SEQ ID NOs: 6 and 13) contain 12 and 14 amino acids, respectively, as seen in Tables 4 and 5 of the specification. All sequences disclosed in the cited reference are larger and as far as Applicants are able to determine do not contain the same amino acid sequence.

For anticipation purposes, Examiner has to show that the structure is the same, that is that the exact amino acid sequences of the current sequences SEQ ID NOs: 6 and 13 are disclosed, but also that their individual function as disclosed and described in the current specification is disclosed by the reference and is the same. Here, the alleged anticipation is not shown and therefore the rejection should be withdrawn.

Moreover, Examiner also rejects claims 1-7 which do not contain any reference to SEQ ID NO : 6 and 13. This rejections is not understood.

Examiner is respectfully requested to reconsider and withdraw this rejection.

Claims 1-6, 12-15 and 23 are rejected under 35 USC 102(b) as being anticipated by WO 90/07525, publication date July 1990. (Please note: the phrase "a recombinant" is being read as a process step that produces a product equivalent to the naturally occurring

purified cytotoxin).

WO 90/07525 discloses the claimed invention directed to a purified, or partially purified *Moraxella bovis* cytotoxin, wherein the cytotoxin, was isolated from *Moraxella bovis* strain Tifton 1 (see page 10, lines 8-10), the same strain from which the coding sequences SEQ ID NO: 1 was determined for the instantly claimed invention.

The cytotoxin/hemolysin was purified from Moraxella bovis cultures that were harvested, centrifuged and filtered (see page 12, lines 3-30, especially paragraph 3) or purified through a series of steps that would result in a cytotoxin equivalent to that which is present in a diafiltrate (see pages 4-5, starting at page 4, line 19 through page 5, line 31; page 17, section D, lines 25-35 and page 18). The purified cytotoxin evidenced leukotoxin activity (see pages 13-16, fractions 4, 20-21), and hemolytic activity fractions 13-17, page 16), with a relative molecular weight of 42 kDa (see page 7, paragraph 2). WO 90/07525 anticipates the instantly claimed invention.

Applicants disagree. Examiner makes much of the process of how to purify the *Moraxella bovis* using the reference cited above, however, nowhere in this reference is even smallest sign of isolating and purifying the cytotoxin having a specific nucleotide or amino acid sequence. With no sequence of the cytotoxin disclosed or even alluded to, the reference does not anticipate the invention. There is no recombinant protein described there and no

sequence disclosed.

Anticipation requires that the reference discloses exactly the same entity and describes it as such and not the entity which might, in Examiner's view, be the same because it was isolated from one of the strains used for isolation and identification and preparation of the current cytotoxins.

Examiner did not show any anticipation of the invention. Rejection should be withdrawn. It is so respectfully requested.

Claims 1-7 are rejected under 35 USC 102(b) as being anticipated by Billson et al, (1994 reference provided in Applicant's US-PTO 1449).

Billson et al discloses the claimed invention directed to a purified, or partially purified produced *Moraxella bovis* cytotoxin (see title and page 72, Table 2) wherein the cytotoxin was purified from culture supernatants, purified by centrifugation (see page 70, col. 1, paragraph 4, bottom of page) and diafiltration (ultrafiltration), and has biological activity of a hemolysin, leukotoxin or corneotoxin with a relative molecular weight of about 65 to 97 kDa (see page 73, col. 1, paragraphs 1-2). The reference by all comparable data, inherently, anticipates the instantly claimed invention.

Applicants disagree. Upon detailed review of the Billson references, it becomes evident that Billson vaccine is prepared form the strain UQV 148 NF haemolytic and not from any of the strains used by the Applicants for the isolation and purification of the current cytotoxins. Additionally, Billson does not list any

sequence which could be compared to the sequences of the current invention. The only recombinant entity (without sequence identification) described by Billson in Table 1 is recombinant Dal 2d pili.

The strains from which the current cytotoxin was isolated are listed on page 12, lines 15-18. The recombinant proteins used for vaccines of the current invention are non-pili proteins.

Claims are not anticipated by Billson reference. Rejection should be withdrawn.

Claims 12-15 and 23-25 are rejected under 35 USC 102(b) as being anticipated by CA2014033-A (publication date October 7, 1990; *Pasteurella haemolytica* leukotoxin).

CA2014033-A disclose the claimed invention directed to an amino acid sequence (claims 12-14), recombinant protein (claim 15), and a composition that comprises a recombinant cytotoxin (claim 23-25) that comprises "an amino acid sequence depicted by SEQ ID NO: 2 or a fragment thereof (se sequence alignment with SEQ ID NO: 1 (which encodes SEQ ID NO: 2), 6 and 13. The reference anticipates the instantly claimed invention.

Applicants disagree. Claims 12, 14 and 15 are amended. Claims 23-25 are canceled. New claims are directed solely to the cytotoxins of the invention thereby eliminating any possibility to include any other cytotoxin, such as cytotoxin produced by *Pasteurella haemolytica*. In view of this amendment the rejection is obviated.

Claims 12, 14-15 and 23 and 25 are rejected under 35 USC 102(b) as being anticipated by US Pat. 5,475,098 (see sequence alignment for SEQ ID NO: 13, *E. coli*.

US Pat. 5,475,098 discloses the claimed invention directed to an amino acid sequence (claims 12, 14) recombinant protein (claim 15) and a composition that comprises a recombinant cytotoxin (claim 23, 25) that comprises "an amino acid sequence depicted by SEQ ID NO: 2 or a fragment thereof wherein the fragment is SEQ ID NO: 13. The reference anticipates the instantly claimed invention.

Applicants disagree. Claims 12, 14 and 15 are amended. Claims 23-25 are canceled. New claims are directed solely to the cytotoxins of the invention thereby eliminating any possibility to include any other cytotoxin, such as cytotoxin produced by *E. coli*. In view of this amendment the rejection is obviated.

Claims 12, 14-15 and 23, 25 are rejected under 35 USC 102(b) a being anticipated by CA2170839 (see sequence alignment for SEQ ID NO: 13, *Actinobacillus pleuropneumonia*).

CA2170839 discloses the claimed invention directed to an amino acid sequence (claims 12, 14) recombinant protein (claim 15), and a composition that comprises a recombinant cytotoxin (claim 23, 25) that comprises "an amino acid sequence depicted by SEQ ID NO: 2 or a fragment thereof wherein the fragment is SEQ ID NO: 13. The reference anticipates the instantly claimed invention.

Applicants disagree. Claims 12, 14 and 15 are amended. Claims 23-25 are canceled. New claims are directed solely to the

cytotoxins of the invention thereby eliminating any possibility to include any other cytotoxin, such as cytotoxin produced by *Actinobacillus pleuropneumonia*. In view of this amendment the rejection is obviated.

Rejections under 35 USC 102 (a) are overcome. Amended claims 1-4, 6, 12-15 and new claims 34-50 are not anticipated and the rejections should be withdrawn and claims allowed to issue. It is so requested.

SUMMARY

In summary, claims are substantially amended or redrafted to meet Examiner's rejections. Rejections under 35 USC 102 are overcome with arguments showing that the invention si not anticipated. It si believed that with this amendment, all rejections are overcome and claims are in conditions for allowance. Notice of allowance is respectfully solicited.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES

1. (Amended) A purified or partially purified *Moraxella bovis* cytotoxin[, produced by a gene comprising] encoded by a DNA sequence SEQ ID NO: 1 deposited at GENBANK database under Accession number AF205359.

2. (Amended) The cytotoxin of claim 1 obtained, purified and isolated from culture supernatants of a[n isolated] cytolytic strain of *Moraxella bovis*.

3. The cytotoxin of claim 2 wherein said cytotoxin is isolated from the culture supernatants [were] purified by centrifugation, filtration, concentration and diafiltration.

4. (Amended) The cytotoxin of claim 3 wherein said cytotoxin is isolated from a [present in the] diafiltered retentate.

6. (Amended) The cytotoxin of claim [5] 4, having a leukotoxic biological activity which causes lysis of bovine neutrophils and lymphoma cells [biologically active as hemolysin, leukotoxin or corneotoxin].

12. (Amended) An isolated amino acid sequence depicted by SEQ ID NO: 2[or a fragment thereof].

13. (Amended) [The] An isolated amino acid sequence [of claim 12 wherein the fragment is] depicted by SEQ ID NO: 6.

14. (Amended) [The] An isolated amino acid sequence [of claim 12 wherein the fragment is] depicted by SEQ ID NO: 13.

15. (Amended) A purified recombinant protein [comprising an] depicted by the amino acid sequence SEQ ID NO: 2 [or a fragment thereof] encoded by the DNA sequence depicted by SEQ ID NO: 1[, or a fragment thereof].

BRIEF DESCRIPTION OF FIGURES

Figure 1 is a graph illustrating neutralization of *M. bovis* leukotoxin in bacterial filter permeates by anti T+ serum.

Figure 2 is an autoradiogram of the pooled and concentrated void volume fraction of diafiltered retentate from T+ and T- cultures chromatographed on a Superose 6HR column.

Figure 3 shows nucleotide (SEQ ID NO: 1) and deduced amino acid (SEQ ID NO: 2) sequences of M. bovis RTXA (MbxA) gene.

Figure 4 shows the alignment of the deduced amino acid sequence of M. bovis RTXA (MbxA) gene (SEQ ID NO: 2) and RTX toxins of *M. (Pasteurella) haemolytica* (LktA) (SEQ ID NO: 3), *A. pleuropneumoniae* (ApxIA) (SEQ ID NO: 4) and *E. coli* (HlyA)(SEQ ID NO: 5).

Figure 5 is an autoradiogram demonstrating MbxA presence in culture supernatants of T+, and Tifton I and absence in T- culture supernatants.

Figure 6 is an autoradiogram of culture supernatants from a Tifton I broth culture at 3, 4, 4.5, 5 and 6 hours time points.

Figure 7 shows the percent neutralization of cytolysis (Figure 7A) and hemolysis (Figure 7B) by non-immune and immune sera alone or preabsorbed with a recombinant expressed carboxy terminus of MbxA.

Figure 8 shows nucleotide (SEQ ID NO: 30) and deduced amino acid (SEQ ID NO: 18) sequences of M. bovis RTXB (MbxB) gene.

points. The blot was probed with rabbit antiserum against the expressed recombinant internal peptide encoded by *MbxA* gene (amino acids 438 through 713). Molecular mass markers 45, 66, 97, 116 and 200 kDa are indicated.

The appearance of proteins that were smaller than the 102 and 105 kDa predicted full length cytotoxin, following 4-6 hours of incubation, as seen in Figure 6, has been attributed to proteolysis. Hemolytic *M. bovis* produces numerous hydrolytic enzymes including C4 esterase, C8 esterase-lipase, C14 lipase, phosphoamidase, phosphatase, leucine and valine aminopeptidases and gelatinase. Proteolysis is believed to account for the difference in mass between the 70 kDa protein selected for amino acid sequencing and the 98.8 kDa molecular mass predicted for full length MbxA. The data indicate that *M. bovis* could also express smaller molecular mass proteins that share epitopes with the internal region MbxA.

To confirm that MbxA encoded the *M. bovis* cytotoxin, neutralization assays were performed with antisera that was preabsorbed with the recombinant expressed carboxy terminus of MbxA (carboxy peptide). Results are seen in Figure 7.

Figure 7 shows percent neutralization (± 1 standard deviation) of cytolysis (Figure 7A) and hemolysis (Figure 7B) by non-immune sera alone or preabsorbed with the carboxy peptide. Cytotoxin was tested for neutralization with nonimmune fetal bovine serum (FBS); with preimmune sera A and B obtained from two rabbits A and B; with immune rabbit anti T+ sera obtained from T+ rabbit polyclonal antisera to culture filtrates from *M. bovis* strain T+; and with immune sera A and B obtained from rabbits A and B vaccinated with the carboxy peptide.

Field trials were performed for testing both the partially purified native and recombinantly produced cytotoxin.

1. Native Cytotoxin Vaccines

M. bovis cytotoxin is a labile protein of which activity rapidly disappears when removed from the bacterial cell. This property previously prevented its complete characterization. To overcome this, a method for partial purification and stabilization of cytotoxin was developed and such partially purified and stabilized cytotoxin was used for preparation of a vaccine for field trials.

In order to determine suitability of such partially purified native *M. bovis* antigen (cytotoxin) for vaccination against *M. bovis*, two field trials were performed where the cytotoxin was administered as a vaccine formulated according to Example 23.

The first field trial was performed on 82 cattle to determine the suitability of the cytotoxin in a vaccine to prevent IBK. In this trial the vaccine was a partially purified cytotoxin formulated either in Quil A adjuvant or in an immunostimulating complex (ISCOM), a matrix that is composed of Quillaja saponins (Quil A), cholesterol, phospholipids and the antigen of interest. ISCOM based vaccines utilizing antigens of Bovine Herpes Virus-1, Bovine Virus Diarrhea Virus and Bovine Leukemia Virus are known to be successful immunogens in cattle.

Prior to the field trial testing the *M. bovis* cytotoxin vaccine, a preliminary study had shown that calves vaccinated with a cytotoxin vaccine developed higher titre IgA concentrations than calves vaccinated with a cytotoxin in oil